MycoKeys 124: 275–290 (2025)
DOI: 10.3897/mycokeys.124.172775

Research Article

New species of *Aspergillus* in sections *Cavernicolarum* and *Nigri* from terrestrial ecosystems of China (Eurotiales, Aspergillaceae)

Lu-Yao Peng[1,2], Wen-Ying Zhuang[1], Xin-Cun Wang[1]

1 *State Key Laboratory of Microbial Diversity and Innovative Utilization, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, China*
2 *University of Chinese Academy of Sciences, Beijing 100049, China*
Corresponding author: Xin-Cun Wang (wangxc@im.ac.cn)

Abstract

Aspergillus species are of great industrial, agricultural, and medicinal importance. During investigations on the biodiversity of Aspergillaceae, two species of *Aspergillus* isolated from soil samples in China were identified as new to science based on sequence analyses and morphological comparisons. *Aspergillus hebeiensis* from a traditional cultural and Buddhist heritage site is the second member of the series *Hainanici* in section *Cavernicolarum* of subgenus *Nidulantes*, while *A. xishuangbannaensis* from a tropical nature reserve is classified in subgenus *Circumdati*, section *Nigri*, series *Japonici*. Detailed descriptions and illustrations of both species are provided, and the ecological functions of their habitats are also discussed.

Key words: Aspergillaceae, biodiversity, Eurotiales, phylogeny, taxonomy

OPEN ACCESS

Academic editor: Xinlei Fan
Received: 22 September 2025
Accepted: 22 October 2025
Published: 3 November 2025

Citation: Peng L-Y, Zhuang W-Y, Wang X-C (2025) New species of *Aspergillus* in sections *Cavernicolarum* and *Nigri* from terrestrial ecosystems of China (Eurotiales, Aspergillaceae). MycoKeys 124: 275–290. https://doi.org/10.3897/mycokeys.124.172775

Copyright: © Lu-Yao Peng et al.
This is an open access article distributed under terms of the Creative Commons Attribution License (Attribution 4.0 International – CC BY 4.0).

Introduction

Species of *Aspergillus* P. Micheli ex Haller are ubiquitous in various environments and have a long history of exploitation by humans. Some species have been used for food fermentations for centuries, especially in East Asia. *Aspergillus oryzae* (Ahlb.) Cohn and *A. sojae* Sakag. & K. Yamada ex Murak. play crucial roles in the production of rice wine, soybean pastes, and soy sauce (Bennett 2009), and *A. niger* Tiegh. is used for fermentations of baijiu (Xu et al. 2022) as well as pu-erh tea (Frisvad et al. 2007). More than 100 years ago, the ability of *A. niger* to produce citric acid was industrially exploited in 1919 (Schuster et al. 2002). *Aspergillus terreus* Thom was the first of the major statins to produce lovastatin, a medicine used to lower cholesterol (Bennett 2009). *Aspergillus niger* and *A. terreus* are also efficient agents for bioleaching of rare earth elements with low environmental impact (Mowafy 2020). *Aspergillus cvjetkovicii* Jurjević et al. can protect against phytopathogens through interspecies chemical signaling in the phyllosphere of rice as a biocontrol agent (Fan et al. 2024). On the other hand, some species pose severe threats to human health. Mycotoxins are produced by certain *Aspergillus* species, causing food contamination, e.g., aflatoxins by *A. flavus* Link and *A. parasiticus* Speare, and ochratoxin A by *A. ochraceus* G. Wilh. and *A. niger* (Xue et al. 2025). *Aspergillus fumigatus*

Fresen. is known as the primary causative agent of aspergillosis, followed by *A. flavus*, *A. niger*, and *A. terreus* (Khan et al. 2024).

The genus *Aspergillus* was originally introduced in 1729 and validated in 1768. It was divided into six subgenera, 27 sections, and 75 series, with 446 species recognized (Houbraken et al. 2020). A new series, *Aspergillus* ser. *Hainanici*, was recently proposed (Wang and Zhuang 2022), and the accepted species of the genus increased to 453 (Visagie et al. 2024). Subsequently, 16 newly described species were added: one each from Europe and North America, two from South America, and the remaining 12 from Asia. *Aspergillus albicolor* D.S. Paiva was reported from Portugal, *A. pseudoalabamensis* Cañete-Gibas et al. from the USA, and *A. alvaroi* J.M.S. Lima et al. and *A. guanovespertilionum* J.M.S. Lima et al. from Brazil. Among the 12 Asian taxa, five were from China (*A. cylindricus* Zhi Y. Zhang et al., *A. doliiformis* Zhi Y. Zhang et al., *A. liaoningensis* C. Liu et al., *A. plumeriae* C. Liu et al., *A. subinflatus* C. Liu et al.), *A. dhakephalkarii* Rajeshk. et al. and *A. patriciawiltshireae* Rajeshk. et al. from India, *A. hubkae* Y.B. Zhou et al. and *A. mahabadiensis* Abdollahz. & O. Ghaderi from Iran, *A. verrucosus* R. Hagiuda & D. Hirose from Japan, *A. ullungdoensis* Hyang B. Lee from South Korea, and *A. halopiscium* V.N. Thanh et al. from Vietnam.

During investigations on the biodiversity of Aspergillaceae in China, two species of *Aspergillus* isolated from soil were identified as new to science based on sequence analyses and morphological comparisons. Detailed descriptions and illustrations are provided.

Materials and methods

Fungal materials

Cultures were isolated from soil samples collected from Hebei and Yunnan provinces, China. Dried cultures were preserved in the Herbarium Mycologicum Academiae Sinicae (HMAS, Beijing, China), and the living ex-type strains were deposited in the China General Microbiological Culture Collection Center (CGMCC, Beijing, China).

Morphological observations

Morphological characteristics were observed and recorded according to standardized methods (Samson et al. 2014). Four standard growth media were adopted: Czapek yeast autolysate agar (CYA; yeast extract, Oxoid, Hampshire, UK), malt extract agar (MEA; Amresco, Solon, OH, USA), yeast extract agar (YES), and potato dextrose agar (PDA). The methods for colonial inoculation, incubation, macroscopic and microscopic examinations, and digital capture followed our previous studies (Wang and Zhuang 2022; Wang et al. 2023; Peng et al. 2025).

DNA extraction, PCR amplification, and sequencing

DNA was extracted from living cultures grown on PDA for 7 days using the Plant Genomic DNA Kit (DP305; TIANGEN Biotech, Beijing, China). Polymerase chain reaction (PCR) amplifications of internal transcribed spacer (ITS), beta-tubulin (BenA), calmodulin (CaM), and RNA polymerase II second-largest

subunit (RPB2) were conducted using routine methods (Samson et al. 2014). The forward and reverse primers used for each locus were as follows: ITS5 (5'-GGA AGT AAA AGT CGT AAC AAG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') for ITS (White et al. 1990); Bt2a (5'-GGT AAC CAA ATC GGT GCT GCT TTC-3') and Bt2b (5'-ACC CTC AGT GTA GTG ACC CTT GGC-3') for BenA (Glass and Donaldson 1995); CMD5 (5'-CCG AGT ACA AGG ARG CCT TC-3') and CMD6 (5'-CCG ATR GAG GTC ATR ACG TGG-3') for CaM (Hong et al. 2005); and 5F (5'-GAY GAY MGW GAT CAY TTY GG-3') and 7CR (5'-CCC ATR GCT TGY TTR CCC AT-3') for RPB2 (Liu et al. 1999). The products were sequenced on an ABI 3730 DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

Phylogenetic analyses

The newly generated forward and reverse sequences in this study were assembled using SeqMan v. 7.1.0 (DNASTAR Inc., Madison, WI, USA). The assembled sequences were deposited in GenBank, with accession numbers shown in bold (Tables 1, 2). The additional sequences used for phylogenetic analyses are also listed. Sequences were aligned using MAFFT v. 7.221 (Katoh and Standley 2013), either as individual single-gene datasets (ITS, BenA, CaM, and RPB2) or concatenated datasets. They were then manually edited and concatenated in BioEdit v. 7.1.10 (Hall 1999) and MEGA v. 11.0.13 (Tamura et al. 2021). Maximum likelihood (ML) analyses were performed using the IQ-TREE web server (Trifinopoulos et al. 2016) with the default automatic substitution model and bootstrap (BP) iteration (1,000 replicates) settings. Bayesian inference (BI) analyses were conducted with MrBayes v. 3.2.7 (Ronquist et al. 2012). Modeltest v. 3.7 (Posada and Crandall 1998) was adopted to determine appropriate nucleotide substitution models and parameters. Four MCMC chains (three heated and one cold) were run for at least 1 million generations, and posterior probability (PP) values were calculated based on the remaining 75% of trees after the burn-in phase. The consensus trees were viewed using FigTree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree, accessed on 28 December 2023).

Table 1. Species and sequences used in the phylogenetic analyses for *Aspergillus* subgenus *Nidulantes* section *Cavernicolarum*.

Series	Species	Strain	Locality	Substrate	ITS	BenA	CaM	RPB2
Cavernicolarum	*A. californicus* Frisvad et al., 2011	CBS 123895 T	USA	chaparral of *Adenostoma fasciculatum*	FJ531153	FJ531180	FJ531128	MN969065
	A. cavernicola Lörinczi, 1969	CBS 117.76 T	Romania	on walls of cave	EF652508	EF652332	EF652420	EF652244
	A. kassunensis Baghd., 1968	CBS 419.69 T	Syria	soil	EF652461	EF652285	EF652373	EF652197
	A. subsessilis Raper & Fennell, 1965	CBS 502.65 T	USA	desert soil	EF652485	EF652309	EF652397	EF652221
Egyptiaci	*A. egyptiacus* Moub. & Mustafa, 1972	CBS 656.73 T	Egypt	sandy soil	EF652504	EF652328	EF652416	EF652240
Hainanici	*A. hainanicus* X.C. Wang & W.Y. Zhuang, 2022	CGMCC 3.20888 T	China: Hainan	sandy soil	OM414846	OM475626	OM475630	OM475634
	***A. hebeiensis* X.C. Wang, L.Y. Peng & W.Y. Zhuang, sp. nov.**	JJJ40-31 T	China: Hebei	soil under *Platycladus orientalis*	**PV883250**	**PV877068**	**PV877070**	**PV877073**
		JJJ40-12	China: Hebei	soil under *Platycladus orientalis*	**PV883251**	n.a.	**PV877071**	**PV877074**

GenBank accession numbers in bold indicate the newly generated sequences. "n.a." is the abbreviation for "not available."

Table 2. Species and sequences used in the phylogenetic analyses for *Aspergillus* subgenus *Circumdati* section *Nigri*.

Series	Species	Strain	Locality	Substrate	ITS	BenA	CaM	RPB2
Japonici	*A. aculeatinus* Noonim et al., 2008	CBS 121060 T	Thailand	dried parchment and green beans of *Coffea arabica*	EU159211	EU159220	EU159241	HF559233
	A. aculeatus Iizuka, 1953	CBS 172.66 T	Japan	unknown	EF661221	HE577806	EF661148	EF661046
	A. brunneoviolaceus Bat. & H. Maia, 1955	CBS 621.78 T	Brazil	gills of Osteichthyes	AJ280003	EF661105	EF661147	EF661045
	A. dhakephalkarii Rajeshk. et al., 2025	NFCCI 5750 T	India	rhizosphere soil associated with *Anthurium andraeanum*	PP741453	PP739067	PP739063	PP739059
	A. floridensis Jurjevic et al., 2012	NRRL 62478 T	USA	air	MN431366	HE984412	HE984429	HE984376
	A. hydei Doilom, 2020	KUMCC 18-0196 T	China: Yunnan	air under *Quercus variabilis*	MT152332	MT161679	MT178247	MT384370
	A. indologenus Frisvad et al., 2011	CBS 114.80 T	India	soil	AJ280005	AY585539	AM419750	HE984366
	A. japonicus Saito, 1906	CBS 114.51 T	unknown	unknown	AJ279985	HE577804	FN594551	MN969079
	A. labruscus Fungaro et al., 2017	IBT 33586 T	Brazil	fruit of *Vitis labrusca*	KU708544	KT986014	KT986008	MN969196
	A. oxumiae C.N. Figueiredo et al., 2020	CCDCA 11546 T	Brazil	soil cultivated with *Agave sisalana*	MN431160	n.a.	MN531842	MN521389
	A. patriciawiltshireae Rajeshk. et al., 2025	NFCCI 5959 T	India	soil	PQ826401	PQ855384	PQ855382	PQ855386
	A. saccharolyticus A. Sørensen et al., 2011	CBS 127449 T	Denmark	toilet seat of treated *Quercus* wood	HM853552	HM853553	HM853554	HF559235
	A. serratalhadensis L.F. Oliveira et al., 2018	URM 7866 T	Brazil	soil	MH169127	LT993222	LT993223	LT995971
	A. trinidadensis Jurjevic et al., 2012	NRRL 62479 T	Trinidad-Tobago	air	MN431380	HE984420	HE984434	HE984379
	A. uvarum G. Perrone et al., 2008	CBS 121591 T	Italy	grape berries	AM745757	AM745751	AM745755	HE984370
	***A. xishuangbannaensis* X.C. Wang, L.Y. Peng & W.Y. Zhuang, sp. nov.**	ZYN05-01 T	China: Yunnan	soil in limestone seasonal rainforest	**PV883252**	**PV877069**	**PV877072**	**PV877075**
Nigri	*A. niger* Tiegh., 1867	CBS 554.65 T	France	unknown	EF661186	EF661089	EF661154	EF661058

GenBank accession numbers in bold indicate the newly generated sequences. "n.a." is the abbreviation for "not available."

Results

To determine the species identities of the investigated strains, the single-gene datasets (ITS, BenA, CaM, and RPB2) and the concatenated three-locus (BenA + CaM + RPB2) dataset were compiled and analyzed. The detailed characteristics of the datasets are listed in Table 3.

In the phylogeny of section *Cavernicolarum* (Fig. 1), the strains JJJ40-12 and JJJ40-31, representing the same species, were located in series *Hainanici* as sister to *Aspergillus hainanicus*. The close relationship between these two species was strongly supported by statistical values, inferred either from the concatenated dataset (MLBP = 100, BIPP = 1.00; Fig. 1) or from the single-gene datasets (MLBP = 100; Suppl. material 1: figs S1–S4).

In the phylogenetic tree of section *Nigri* series *Japonici* (Fig. 2), strain ZYN05-01 was grouped into a small clade associated with the following species: *A. japonicus*, *A. indologenus*, and *A. uvarum*. A similar tree topology was shown in the BenA analysis (Suppl. material 1: fig. S5), but it differed somewhat from the other two single-gene inferences. Strain ZYN05-01 clustered with *A. uvarum* in the CaM tree (MLBP = 82; Suppl. material 1: fig. S6), but it was closely related to *A. japonicus* in the RPB2 phylogeny (MLBP = 94; Suppl. material 1: fig. S7).

Table 3. Detailed characteristics of the datasets.

Dataset	Gene fragment	No. of seq.	Length of alignment (bp)	No. of variable sites	No. of parsimony-informative sites	Model for BI
Cavernicolarum	ITS	8	548	64	44	
	BenA	7	471	150	83	
	CaM	8	553	204	129	
	RPB2	8	1071	241	151	
	BenA+CaM+RPB2	8	2095	595	363	TIMef+I
Japonici	BenA	16	530	235	108	
	CaM	17	580	245	128	
	RPB2	17	1052	273	155	
	BenA+CaM+RPB2	17	2162	753	391	TrN+I+G

Abbreviations of the model: TIMef+I (equal-frequency transition model with invariant sites), TrN+I+G (Tamura–Nei model with invariant sites and gamma distribution).

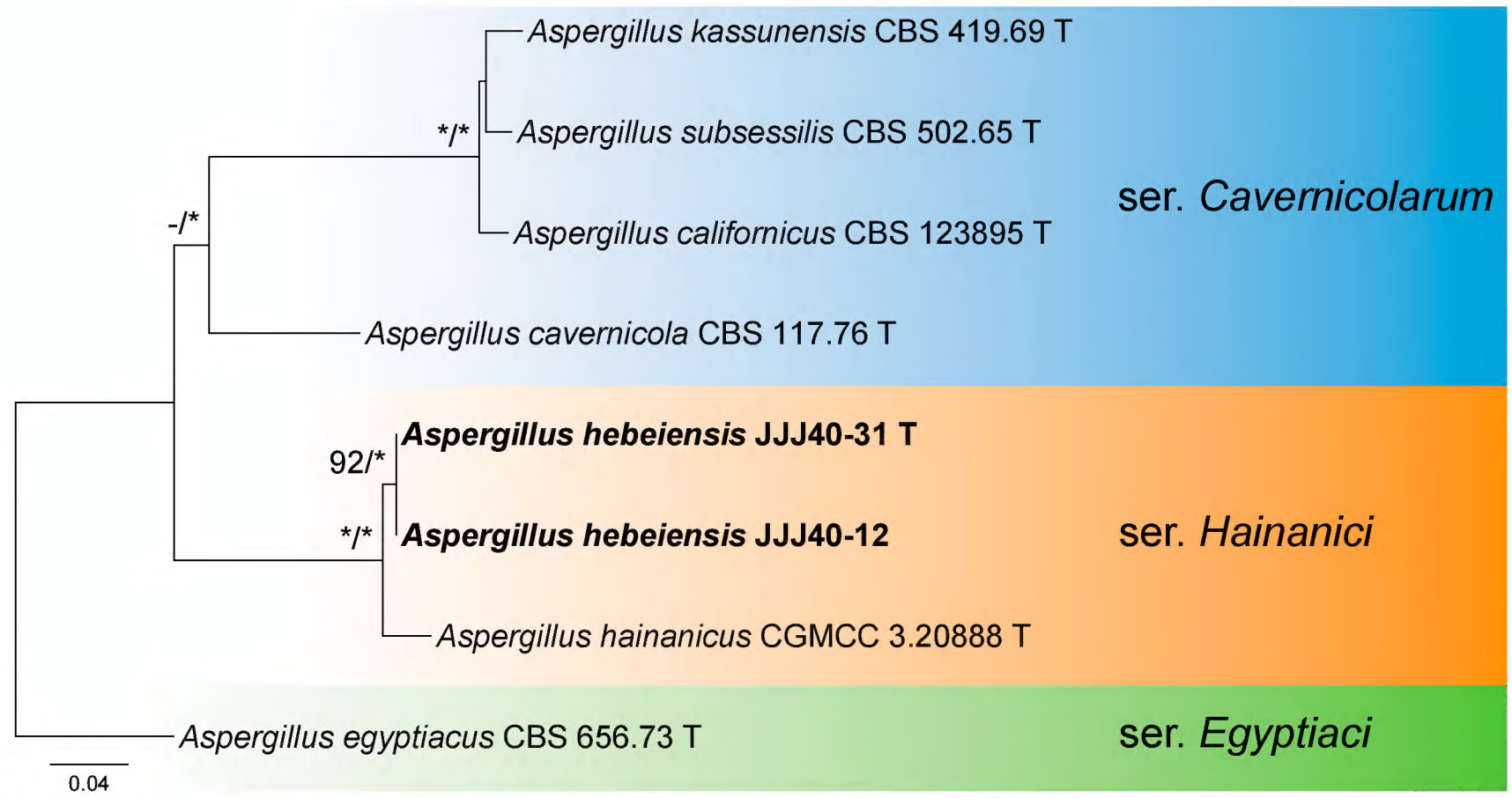

Figure 1. Maximum likelihood phylogeny of *Aspergillus* subgenus *Nidulantes* section *Cavernicolarum* inferred from the combined BenA, CaM, and RPB2 dataset. Bootstrap values ≥ 70% (left) or posterior probability values ≥ 0.95 (right) are indicated at nodes. Asterisks denote 100% bootstrap or 1.00 posterior probability.

Taxonomy

Aspergillus hebeiensis **X.C. Wang, L.Y. Peng & W.Y. Zhuang, sp. nov.**

Fig. 3

Fungal Names: FN 572829

Etymology. The specific epithet refers to the type locality.

In *Aspergillus* subgenus *Nidulantes* section *Cavernicolarum* series *Hainanici*.

Typification. China • Hebei Province, Handan City, Fengfeng Mining District, Xiangtangshan Caves, one of the First Batch of Key Cultural Relics Units under National Protection of China, Northern Xiangtangshan, 36°32'2"N, 114°9'40"E, in soil under *Platycladus orientalis* (L.) Franco, 17 July 2023, Xin-Cun Wang, culture, Lu-Yao Peng, JJJ40-31 (holotype HMAS 354080, ex-type strain CGMCC 3.29151).

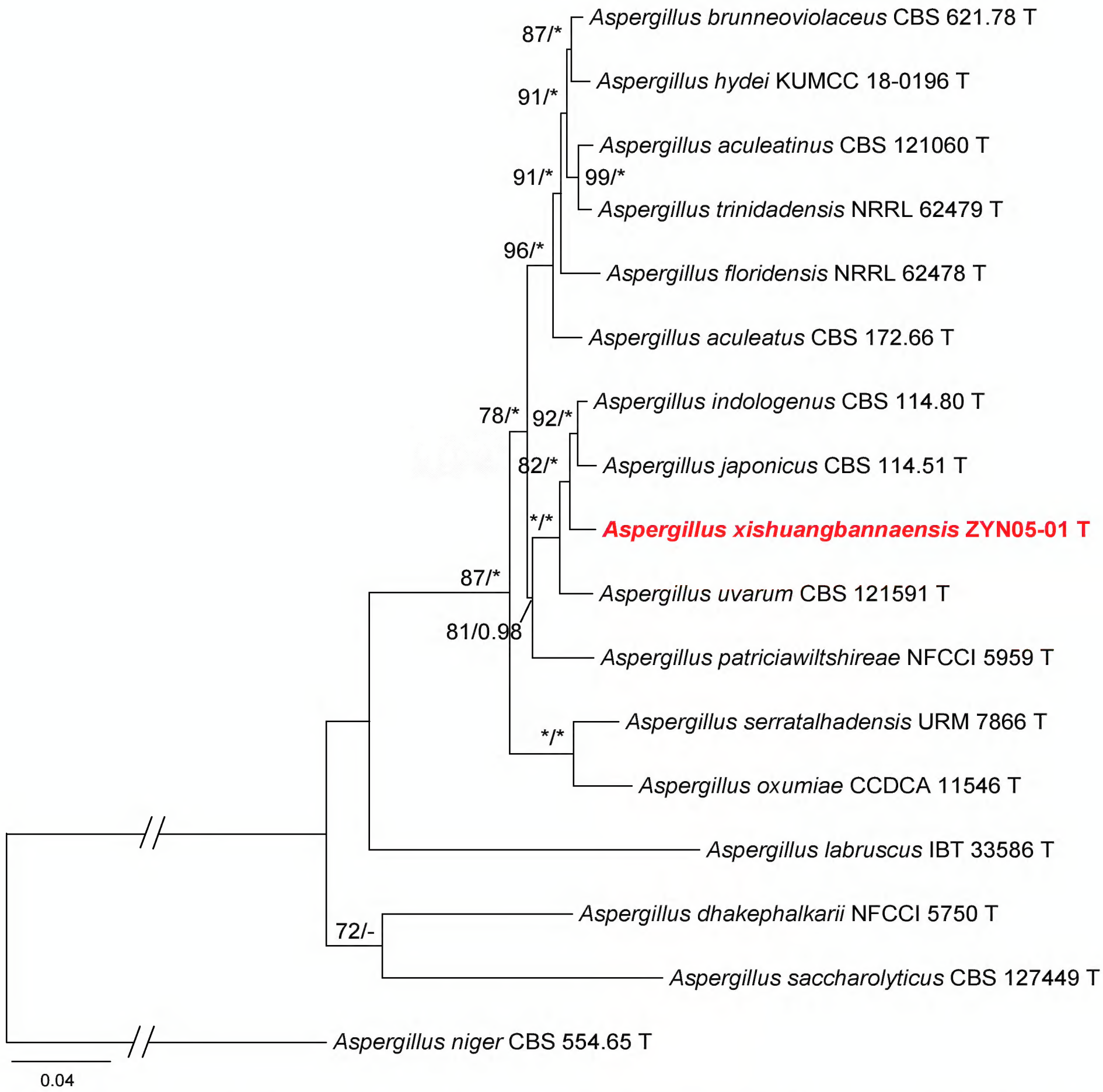

Figure 2. Maximum likelihood phylogeny of *Aspergillus* subgenus *Circumdati* section *Nigri* series *Japonici* inferred from the combined BenA, CaM, and RPB2 dataset. Bootstrap values ≥ 70% (left) or posterior probability values ≥ 0.95 (right) are indicated at nodes. Asterisks denote 100% bootstrap or 1.00 posterior probability.

DNA barcodes. ITS PV883250, BenA PV877068, CaM PV877070, RPB2 PV877073.

Colony diam. 7 days, 25 °C (unless stated otherwise): CYA 15–17 mm; CYA 37 °C 12–15 mm; MEA 19–20 mm; YES 18–19 mm; PDA 16–17 mm.

Colony characteristics. On CYA 25 °C, 7 days: Colonies nearly circular, slightly concave at centers; margins moderately wide, entire; mycelia white and then yellow; texture velutinous; sporulation sparse; conidia *en masse* greyish; soluble pigments absent; exudates absent; reverse buff to yellow brown.

On CYA 37 °C, 7 days: Colonies nearly circular or irregular, slightly protuberant at centers; margins narrow to moderately wide, fimbriate; mycelia white and

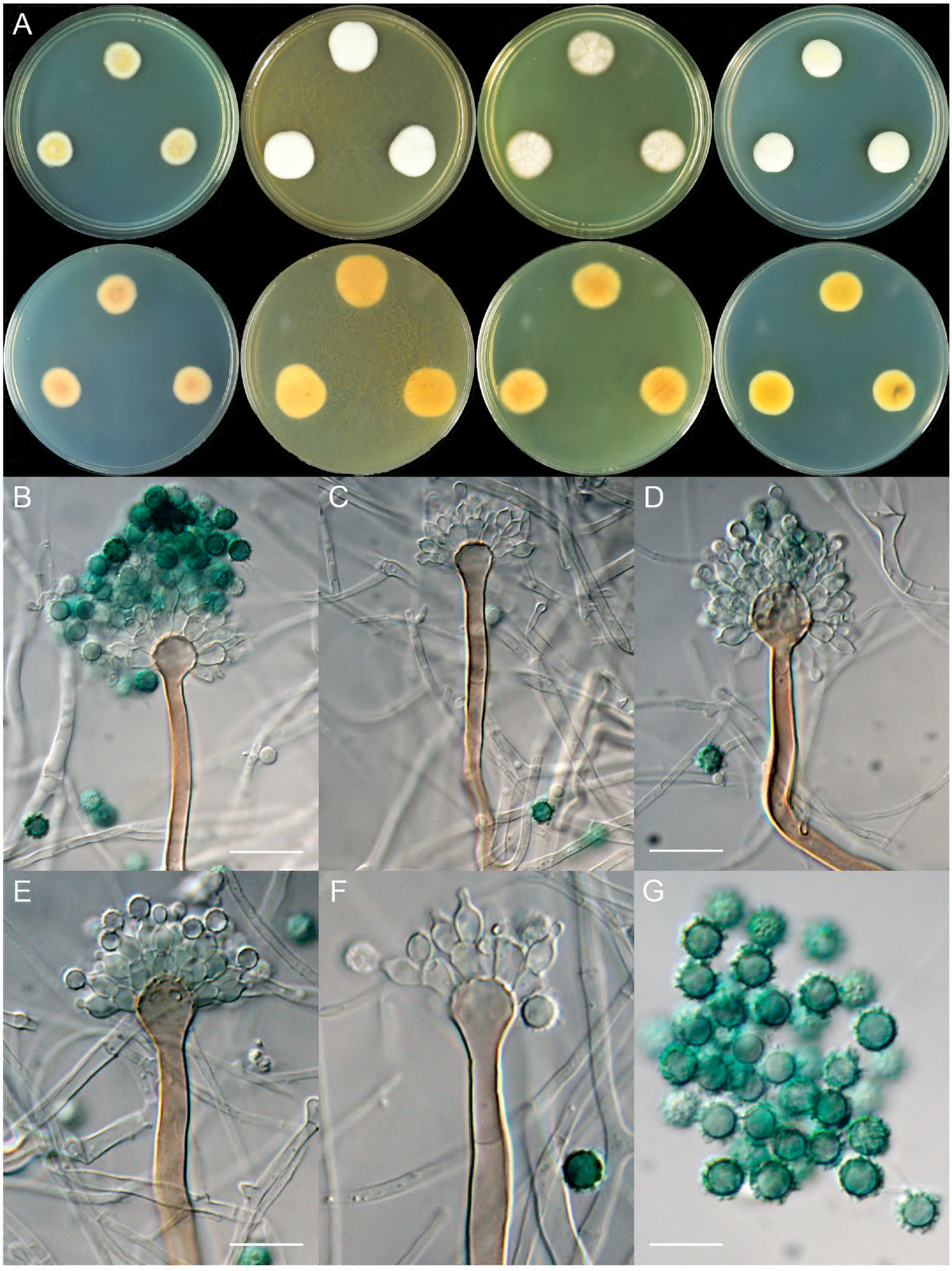

Figure 3. *Aspergillus hebeiensis* (JJJ40-31). **A.** Colonies: top row left to right, obverse CYA, MEA, YES, and PDA; bottom row left to right, reverse CYA, MEA, YES, and PDA; **B–F.** Conidiophores; **G.** Conidia. Scale bars: 17.5 µm (**B, C**); 15 µm (**D**); 12.5 µm (**E**); 10 µm (**G, F**).

then buff; texture velutinous; sporulation sparse; conidia *en masse* brownish; soluble pigments absent; exudates absent; reverse buff to yellow brown.

On MEA 25 °C, 7 days: Colonies irregular, protuberant; margins narrow, entire; mycelia white and then cream; texture velutinous; sporulation sparse; conidia *en masse* creamish; soluble pigments absent; exudates absent; reverse buff to yellow brown.

On YES 25 °C, 7 days: Colonies nearly circular, concave at centers, radially sulcate; margins narrow, entire; mycelia pale; texture velutinous; sporulation sparse; conidia *en masse* greyish; soluble pigments absent; exudates absent; reverse yellow brown to orange brown.

On PDA 25 °C, 7 days: Colonies nearly circular, protuberant; margins narrow, entire; mycelia white and then cream; texture velutinous; sporulation sparse; conidia *en masse* creamish to brownish; soluble pigments yellow; exudates absent; reverse buff to yellow brown, occasionally with dark brown sectors.

Micromorphology. Conidial heads radiate; stipes short, 65–110 (–140) × 4.0–7.0 µm, not septate, walls thick, smooth, brown; vesicles 8.5–13 × 8.5–13 µm, subglobose to globose; biseriate; metulae 5.0–8.5 × 3.5–6.5 µm, cylindrical to obovate, covering almost a half to two-thirds surface of the vesicle; phialides 5.5–8.0 × 3.5–4.0 µm, flask-shaped; conidia 6.0–7.5 µm, subglobose, vivid green, strongly echinulate.

Additional strain examined. CHINA • Hebei Province, Handan City, Fengfeng Mining District, Xiangtangshan Caves, one of the First Batch of Key Cultural Relics Units under National Protection of China, Northern Xiangtangshan, 36°32'2"N, 114°9'40"E, in soil under *Platycladus orientalis* (L.) Franco, 17 July 2023, Xin-Cun Wang, culture, Yi-Fan Wang, JJJ40-12.

Notes. This species is the second member of series *Hainanici* and sister to *A. hainanicus* (Fig. 1; Suppl. material 1: figs S1–S4). It differs from the latter by 29 bp for BenA (93.76% sequence identity), 16 bp for CaM (97.03%), and 21 bp for RPB2 (98.04%). Morphologically, although both species have short stipes, biseriate conidiophores, and strongly echinulate conidia, the new species is easily distinguished from *A. hainanicus* by growth on CYA at 37 °C, vivid green, and smaller conidia (6.0–7.5 vs. 6.0–9.5 µm, Table 4).

Aspergillus xishuangbannaensis **X.C. Wang, L.Y. Peng & W.Y. Zhuang, sp. nov.**

Fig. 4

Fungal Names: FN 572923

Etymology. The specific epithet refers to the type locality.

In *Aspergillus* subgenus *Circumdati* section *Nigri* series *Japonici*.

Typification. CHINA • Yunnan Province, Xishuangbanna Dai Autonomous Prefecture, Mengla County, Menglun Town, Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences, Green Stone Forest, 21°54'39"N, 101°17'00"E, in soil of limestone seasonal rainforest, 28 May 2024, Zhao-Qing Zeng, culture, Xiao Mou, ZYN05-01 (holotype HMAS 354081, ex-type strain CGMCC 3.29152).

DNA barcodes. ITS PV883252, BenA PV877069, CaM PV877072, RPB2 PV877075.

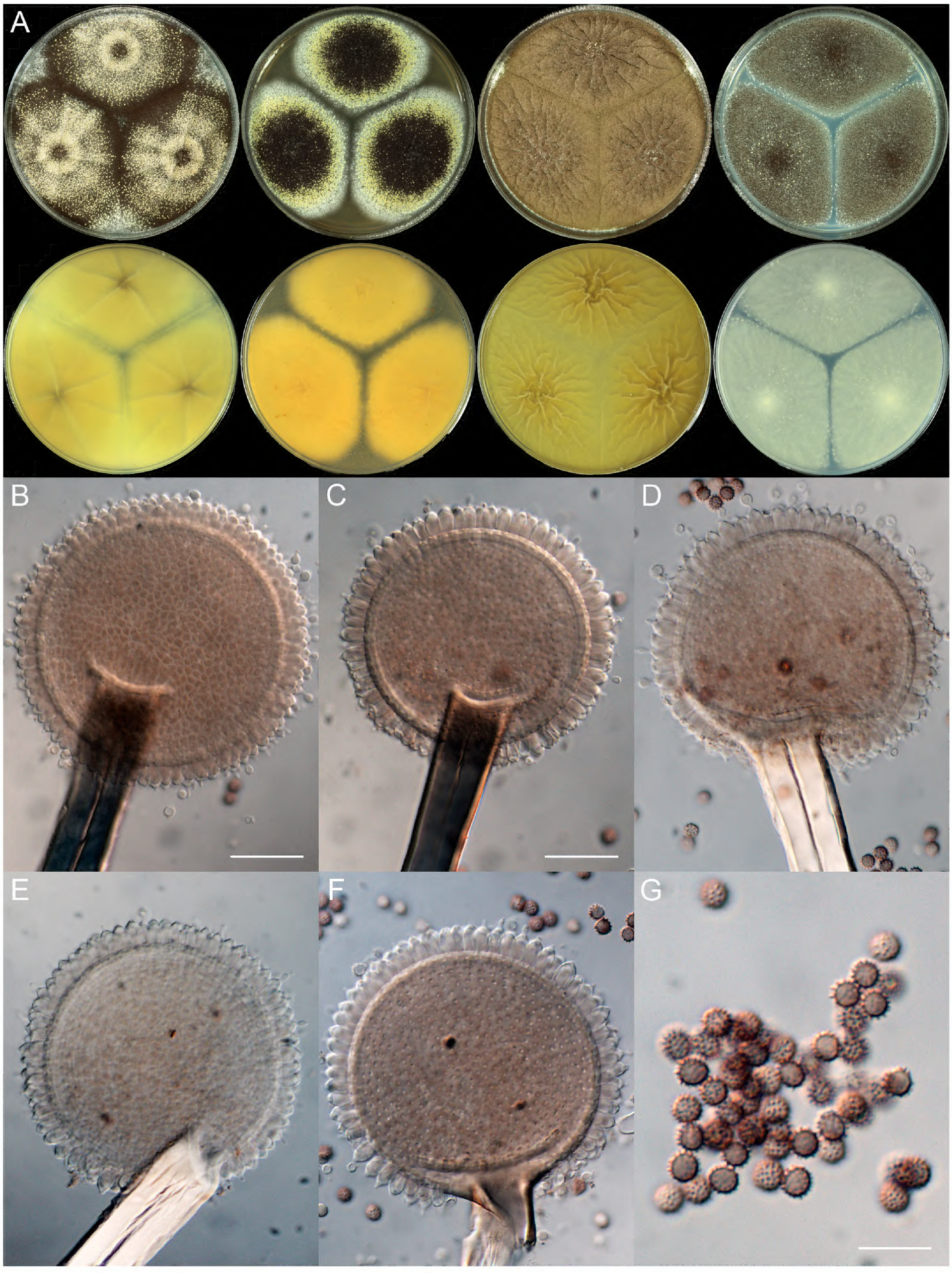

Figure 4. *Aspergillus xishuangbannaensis* (ZYN05-01). **A.** Colonies: top row left to right, obverse CYA, MEA, YES, and PDA; bottom row left to right, reverse CYA, MEA, YES, and PDA; **B–F.** Conidiophores; **G.** Conidia. Scale bars: 22.5 µm (**B**); 20 µm (**C–F**); 10 µm (**G**).

Table 4. Morphological comparisons of the new species and their closely related species.

Series	Species	CYA 25 °C (mm)	CYA 37 °C (mm)	MEA (mm)	YES (mm)	Vesicles (μm)	Conidia shape	Conidia color	Conidia wall	Conidia size (μm)	Reference
Hainanici	***A. hebeiensis***	15–17	12–15	19–20	18–19	8.5–13 × 8.5–13	subglobose	vivid green	strongly echinulate	6.0–7.5	This study
	A. hainanicus	18–20	no growth	16–17	21–22	7.5–13 × 9.0–13	subglobose	hyaline	strongly echinulate	6.0–9.5	Wang and Zhuang 2022
Japonici	***A. xishuangbannaensis***	64–70	35–57	55–60	68–70	62–80 × 60.5–77.5	subglobose	brown	echinulate	4.5–5.0 × 4.0–5.0	This study
	A. japonicus	60–70	20–50	60–70	n.a.	20–35	globose to subglobose	brown	echinulate	3.5–5.0	Klich 2002; Samson et al. 2007
	A. uvarum	90	16–22	90	n.a.	20–30	globose to subglobose	brown to black	conspicuously spinose	3.0–4.0	Perrone et al. 2008
	A. indologenus	63–70	n.a.	57–70	76–80	20–45	globose	brown	coarsely roughened to echinulate	3.0–4.0	Varga et al. 2011

'n.a.' is the abbreviation of 'not available.'

Colony diam. 7 days, 25 °C (unless stated otherwise): CYA 64–70 mm; CYA 37 °C 35–57 mm; MEA 55–60 mm; YES 68–70 mm; PDA 66–70 mm.

Colony characters. CYA 25 °C, 7 d: Colonies deep, plane, radially sulcate; margins entire; mycelium white; texture velutinous; sporulation moderately dense; conidia *en masse* dark brown; soluble pigments absent; exudates absent; sclerotia yellow, abundant; reverse yellow brown.

CYA 37 °C, 7 d: Colonies deep, protuberant, radially sulcate; margins narrow, entire; mycelium white; texture velutinous; sporulation dense; conidia *en masse* dark brown; soluble pigments absent; exudates tiny; reverse yellow brown to greyish black.

MEA 25 °C, 7 d: Colonies plane; margins moderately wide, entire; mycelium white; texture velutinous; sporulation dense; conidia *en masse* blackish brown; soluble pigments absent; exudates absent; sclerotia yellow, abundant; reverse yellow brown.

YES 25 °C, 7 d: Colonies deep, radially sulcate; margins entire; mycelium white; texture velutinous; sporulation very dense; conidia *en masse* purplish brown; soluble pigments absent; exudates absent; sclerotia yellow or white; reverse yellow brown.

PDA 25 °C, 7 d: Colonies plain; margins narrow, entire; mycelium white; texture velutinous; sporulation dense; conidia *en masse* dark brown; soluble pigments absent; exudates absent; sclerotia yellow; reverse white.

Micromorphology. Conidial heads radiate; stipes 400–975 × 12–21.5 μm, not septate, walls thick, smooth, hyaline, brownish or black; vesicles 62–80 × 60.5–77.5 μm, globose or subglobose; uniseriate; phialides 6.5–8.5 × 3.5–5.5 μm, flask-shaped and cover the entire surface of the vesicle; conidia 4.5–5.0 × 4.0–5.0 μm, subglobose, echinulate, light brown to dark brown when mature.

Notes. *Aspergillus xishuangbannaensis* is molecularly and morphologically differentiated from its closely related sisters: *A. japonicus*, *A. uvarum*, and *A. indologenus*. For the BenA gene, it differs from *A. japonicus* by 13 bp (97.27% sequence identity), from *A. uvarum* by 16 bp (96.26%), and from *A. indologenus* by 14 bp (97.06%); for the CaM region, it differs from *A. japonicus* by 20 bp (95.97%), from *A. uvarum* by 15 bp (96.98%), and from *A. indologenus* by

17 bp (96.72%); and for the RPB2 fragment, it differs from *A. japonicus* by five bp (99.50%), from *A. uvarum* by 19 bp (98.19%), and from *A. indologenus* by seven bp (99.33%). Although species of this series are similar in gross morphology, the new species can be easily separated from its sisters by much larger vesicles (Table 4). Additionally, sclerotia were not observed in *A. indologenus*, while white to cream sclerotia were often produced by *A. japonicus*, yellow ones in the new species, and dark brown to black ones in *A. uvarum* (Klich 2002; Samson et al. 2007; Perrone et al. 2008; Varga et al. 2011).

Discussion

In our previous study, four new species of *Aspergillus* were described from the Xisha Islands in the South China Sea (Wang and Zhuang 2022). That was the first report of a new species of the genus from Chinese tropical islands, suggesting that the biodiversity level of *Aspergillus* might be underestimated in marine environments. *Aspergillus liaoningensis*, *A. plumeriae*, and *A. subinflatus* were later found in tidal flat sediments (Liu et al. 2023), and *A. halopiscium* occurred in the dried marine anchovy *Stolephorus commersonnii* Lacepède (Crous et al. 2025). These accumulated findings reveal that marine environments are biodiversity hotspots for the genus.

In contrast, the two new species discovered in this study are both from terrestrial ecosystems, including a cultural heritage site and a nature reserve. This suggests that terrestrial ecosystems also should not be overlooked in biodiversity investigations.

The Xiangtangshan Caves, located in the southern part of Hebei Province in North China, are a famous cultural treasure containing 16 caves adorned with over 4,000 Buddhist sculptures dating back more than 1,400 years. The new species *A. hebeiensis* was isolated from a soil sample collected in this area. Chinese Buddhist temples have been shown to play an important role in preserving regional biodiversity (Wang et al. 2020; Huang et al. 2025). Further explorations in these traditional cultural and religious areas should be emphasized.

Caves are another biodiversity hotspot, and several *Aspergillus* species were originally reported from them. *Aspergillus alvaroi* was isolated from sediment in a Brazilian cave, *A. guanovespertilionum* from hematophagous bat guano (Lima et al. 2024), *A. lebretii* V.C.S. Alves et al. from cave air (Alves et al. 2022), *A. okavangoensis* Visagie & Nkwe from bat guano-contaminated soil in a Botswana cave (Visagie et al. 2021), and *A. limoniformis* Z.F. Zhang & L. Cai, *A. phialiformis* Z.F. Zhang & L. Cai, and *A. phialosimplex* Z.F. Zhang & L. Cai from dung of Chiroptera, rock, and plant debris in Chinese caves, respectively (Zhang et al. 2021).

The tropical seasonal rain forest in Xishuangbanna is one of the most species-rich forest ecosystems in China. This area is also one of the biodiversity hotspots in the world. Xishuangbanna National Nature Reserve is one of China's first nature reserves, established in 1958. It consists of five geographically disconnected sub-reserves: Mangao Reserve, Mengla Reserve, Menglun Reserve, Mengyang Reserve, and Shangyong Reserve. Xishuangbanna Tropical Botanical Garden, located in Menglun Town and covering 1,125 hectares, is home to more than 14,000 plant species, including rare orchids and ancient cycads. Many fungal species were first described from Xishuangbanna, e.g., *Chloridium xishuangbannaense* W.P. Wu & Y.Z. Diao (Wu and Diao 2022),

Talaromyces bannicus L. Wang (Wei et al. 2021), and *Trichoderma bannaense* Kai Chen & W.Y. Zhuang (Chen and Zhuang 2017). However, no new species of Aspergillaceae had been recorded from this district. *Aspergillus xishuangbannaensis*, described here, was isolated from a soil sample collected in this area. More surveys are urgently needed to explore the species diversity of this group in diverse terrestrial ecosystems in China.

Acknowledgements

The authors thank Dr. Zhao-Qing Zeng and Mr. Xiao Mou of the same institute for providing the strain of *A. xishuangbannaensis*.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

Funding

This project was supported by the National Natural Science Foundation of China (32270008).

Author contributions

Conceptualization: XCW. Data curation: LYP, WYZ, XCW. Formal analysis: LYP, XCW. Funding acquisition: XCW, WYZ. Investigation: XCW. Methodology: XCW. Project administration: WYZ, XCW. Resources: WYZ, XCW. Software: XCW. Supervision: XCW, WYZ. Validation: WYZ, XCW. Visualization: LYP, XCW. Writing - original draft: XCW. Writing - review and editing: XCW, WYZ.

Author ORCIDs

Lu-Yao Peng https://orcid.org/0009-0004-9179-4826
Wen-Ying Zhuang https://orcid.org/0000-0002-1162-9678
Xin-Cun Wang https://orcid.org/0000-0003-1780-7735

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Alves VCS, Lira RA, Lima JMS, Barbosa RN, Bento DM, Barbier E, Bernard E, Souza-Motta CM, Bezerra JDP (2022) Unravelling the fungal darkness in a tropical cave: Richness and the description of one new genus and six new species. Fungal Systematics and Evolution 10: 139–167. https://doi.org/10.3114/fuse.2022.10.06

Bennett JW (2009) *Aspergillus*: A primer for the novice. Medical Mycology 47(Suppl 1): S5–S12. https://doi.org/10.1080/13693780802712515

Chen K, Zhuang WY (2017) Discovery from a large-scaled survey of *Trichoderma* in soil of China. Scientific Reports 7: 9090. https://doi.org/10.1038/s41598-017-07807-3

Crous PW, Catcheside DEA, Catcheside PS, Alfenas AC, Alfenas RF, Barreto RW, Lebel T, Balashov S, Broadbridge J, Jurjevic Z, De la Pena-Lastra S, Hoffmann R, Mateos A, Riebesehl J, Shivas RG, Soliz Santander FF, Tan YP, Altes A, Bandini D, Carriconde F, Cazabonne J, Czachura P, Gryta H, Eyssartier G, Larsson E, Pereira OL, Rigueiro-Rodriguez A, Wingfield MJ, Ahmad W, Bibi S, Denman S, Esteve-Raventos F, Hussain S, Illescas T, Luangsa-Ard JJ, Moller L, Mombert A, Noisripoom W, Olariaga I, Pancorbo F, Paz A, Piatek M, Polman-Short C, Suarez E, Afshan NS, Ali H, Arzanlou M, Ayer F, Barratt J, Bellanger JM, Bidaud A, Bishop-Hurley SL, Bohm M, Bose T, Campo E, Chau NB, Colak OF, Cordeiro TRL, Cruz MO, Custodio FA, Couceiro A, Darmostuk V, Dearnaley JDW, de Azevedo Santiago A, de Freitas LWS, Yanez-Morales MJ, Domnauer C, Dentinger B, Dhileepan K, De Souza JT, Dovana F, Eberhardt U, Eisvand P, Erhard A, Fachada V, Garcia-Martin A, Groenewald M, Hammerbacher A, Harms K, Haroon S, Haqnawaz M, Henriques S, Hernandez AJ, Jacobus LM, Jaen-Contreras D, Jangsantear P, Kaygusuz O, Knoppersen R, Kumar TKA, Lynch MJ, Mahiques R, Maraia GL, Marbach PAS, Mehrabi-Koushki M, Miller PR, Mongkolsamrit S, Moreau PA, Oberlies NH, Oliveira JA, Orlovich D, Perez-Mendez AS, Pinto A, Raja HA, Ramirez GH, Raphael B, Rodrigues A, Rodrigues H, Ramos DO, Safi A, Sarwar S, Saar I, Sanchez RM, Santana JS, Scrace J, Sales LS, Silva LNP, Stryjak-Bogacka M, Tacconi A, Thanh VN, Thomas A, Thuy NT, Toome M, Valdez-Carrazco JM, van Vuuren NI, Vasey J, Vauras J, Vila-Vicosa C, Villarreal M, Visagie CM, Vizzini A, Whiteside EJ, Groenewald JZ (2025) Fungal Planet description sheets: 1781–1866. Persoonia 54: 327–587. https://doi.org/10.3114/persoonia.2025.54.10

Fan XY, Matsumoto H, Xu HR, Fang HD, Pan QQ, Lv TX, Zhan CF, Feng XX, Liu XY, Su DR, Fan MY, Ma ZH, Berg G, Li SJ, Cernava T, Wang MC (2024) *Aspergillus cvjetkovicii* protects against phytopathogens through interspecies chemical signalling in the phyllosphere. Nature Microbiology 9: 2862–2876. https://doi.org/10.1038/s41564-024-01781-z

Frisvad JC, Larsen TO, de Vries R, Meijer M, Houbraken J, Cabanes FJ, Ehrlich K, Samson RA (2007) Secondary metabolite profiling, growth profiles and other tools for species recognition and important *Aspergillus* mycotoxins. Studies in Mycology 59: 31–37. https://doi.org/10.3114/sim.2007.59.04

Glass NL, Donaldson GC (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. Applied and Environmental Microbiology 61: 1323–1330. https://doi.org/10.1128/aem.61.4.1323-1330.1995

Hall TA (1999) BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41: 95–98.

Hong SB, Go SJ, Shin HD, Frisvad JC, Samson RA (2005) Polyphasic taxonomy of *Aspergillus fumigatus* and related species. Mycologia 97: 1316–1329. https://doi.org/10.3852/mycologia.97.6.1316

Houbraken J, Kocsube S, Visagie CM, Yilmaz N, Wang XC, Meijer M, Kraak B, Hubka V, Bensch K, Samson RA, Frisvad JC (2020) Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): An overview of families, genera, subgenera, sections, series and species. Studies in Mycology 95: 5–169. https://doi.org/10.1016/j.simyco.2020.05.002

Huang L, Tian L, Huang L, Jin C, Hu S, Zhang Z, Yan E, Jim CY, Yang Y, Lindenmayer DB, Tang Z (2025) Religious temples are long-term refuges for old trees in human-dominated landscapes in China. Current Biology 35: 2994–3000. https://doi.org/10.1016/j.cub.2025.05.030

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Khan S, Bilal H, Shafiq M, Zhang D, Awais M, Chen C, Khan MN, Wang Q, Cai L, Islam R, Zeng Y (2024) Distribution of *Aspergillus* species and risk factors for aspergillosis in mainland China: A systematic review. Therapeutic Advances in Infectious Disease 11: 20499361241252537. https://doi.org/10.1177/20499361241252537

Klich MA (2002) Identification of common *Aspergillus* species. Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, 116 pp.

Lima JMS, Barbosa RN, Bento DM, Barbier E, Bernard E, Bezerra JDP, Souza-Motta CM (2024) *Aspergillus*, *Penicillium*, and *Talaromyces* (Eurotiales) in Brazilian caves, with the description of four new species. Fungal Systematics and Evolution 14: 89–107. https://doi.org/10.3114/fuse.2024.14.06

Liu YJ, Whelen S, Hall BD (1999) Phylogenetic relationships among ascomycetes: Evidence from an RNA polymerse II subunit. Molecular Biology and Evolution 16: 1799–1808. https://doi.org/10.1093/oxfordjournals.molbev.a026092

Liu C, Wang XC, Yu ZH, Zhuang WY, Zeng ZQ (2023) Seven new species of Eurotiales (Ascomycota) isolated from tidal flat sediments in China. Journal of Fungi (Basel, Switzerland) 9: 960. https://doi.org/10.3390/jof9100960

Mowafy AM (2020) Biological leaching of rare earth elements. World Journal of Microbiology & Biotechnology 36: 61. https://doi.org/10.1007/s11274-020-02838-x

Peng LY, Wang XC, Gafforov Y, Zhuang WY (2025) Seven new series and four new species in sections *Subinflati* and *Trachyspermi* of *Talaromyces* (Trichocomaceae, Eurotiales). Journal of Fungi (Basel, Switzerland) 11: 508. https://doi.org/10.3390/jof11070508

Perrone G, Varga J, Susca A, Frisvad JC, Stea G, Kocsube S, Toth B, Kozakiewicz Z, Samson RA (2008) *Aspergillus uvarum* sp. nov., an uniseriate black *Aspergillus* species isolated from grapes in Europe. International Journal of Systematic and Evolutionary Microbiology 58: 1032–1039. https://doi.org/10.1099/ijs.0.65463-0

Posada D, Crandall KA (1998) MODELTEST: Testing the model of DNA substitution. Bioinformatics (Oxford, England) 14: 817–818. https://doi.org/10.1093/bioinformatics/14.9.817

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Hohna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Samson RA, Noonim P, Meijer M, Houbraken J, Frisvad JC, Varga J (2007) Diagnostic tools to identify black aspergilli. Studies in Mycology 59: 129–145. https://doi.org/10.3114/sim.2007.59.13

Samson RA, Visagie CM, Houbraken J, Hong SB, Hubka V, Klaassen CH, Perrone G, Seifert KA, Susca A, Tanney JB, Varga J, Kocsube S, Szigeti G, Yaguchi T, Frisvad JC (2014) Phylogeny, identification and nomenclature of the genus *Aspergillus*. Studies in Mycology 78: 141–173. https://doi.org/10.1016/j.simyco.2014.07.004

Schuster E, Dunn-Coleman N, Frisvad JC, Van Dijck PW (2002) On the safety of *Aspergillus niger* – a review. Applied Microbiology and Biotechnology 59: 426–435. https://doi.org/10.1007/s00253-002-1032-6

Tamura K, Stecher G, Kumar S (2021) MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. https://doi.org/10.1093/molbev/msab120

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44: W232–235. https://doi.org/10.1093/nar/gkw256

Varga J, Frisvad JC, Kocsube S, Brankovics B, Toth B, Szigeti G, Samson RA (2011) New and revisited species in *Aspergillus* section *Nigri*. Studies in Mycology 69: 1–17. https://doi.org/10.3114/sim.2011.69.01

Visagie CM, Goodwell M, Nkwe DO (2021) *Aspergillus* diversity from the Gcwihaba Cave in Botswana and description of one new species. Fungal Systematics and Evolution 8: 81–89. https://doi.org/10.3114/fuse.2021.08.07

Visagie CM, Yilmaz N, Kocsubé S, Frisvad JC, Hubka V, Samson RA, Houbraken J (2024) A review of recently introduced *Aspergillus*, *Penicillium*, *Talaromyces* and other Eurotiales species. Studies in Mycology 107: 1–66. https://doi.org/10.3114/sim.2024.107.01

Wang XC, Zhuang WY (2022) New species of *Aspergillus* (Aspergillaceae) from tropical islands of China. Journal of Fungi (Basel, Switzerland) 8: 225. https://doi.org/10.3390/jof8030225

Wang X, Jin C, Huang L, Zhou L, Zheng M, Qian S, Yang Y (2020) Plant diversity and species replacement in Chinese Buddhist temples. Shengwu Duoyangxing 28: 668–677. https://doi.org/10.17520/biods.2019392

Wang XC, Zhang ZK, Zhuang WY (2023) Species diversity of *Penicillium* in Southwest China with discovery of forty-three new species. Journal of Fungi (Basel, Switzerland) 9: 1150.

Wei S, Xu X, Wang L (2021) Four new species of *Talaromyces* section *Talaromyces* discovered in China. Mycologia 113: 492–508. https://doi.org/10.1080/00275514.2020.1853457

White TJ, Bruns TD, Lee SB, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (Eds) PCR Protocols: a guide to methods and applications. Academic Press, New York, USA, 315–322. https://doi.org/10.1016/B978-0-12-372180-8.50042-1

Wu WP, Diao YZ (2022) Anamorphic chaetosphaeriaceous fungi from China. Fungal Diversity 116: 1–546. https://doi.org/10.1007/s13225-022-00509-w

Xu Y, Huang H, Lu H, Wu M, Lin M, Zhang C, Zhao Z, Li W, Zhang C, Li X, Sun B (2022) Characterization of an *Aspergillus niger* for efficient fatty acid ethyl ester synthesis in aqueous phase and the molecular mechanism. Frontiers in Microbiology 12: 820380. https://doi.org/10.3389/fmicb.2021.820380

Xue M, Qu Z, Moretti A, Logrieco AF, Chu H, Zhang Q, Sun C, Ren X, Cui L, Chen Q, An Y, Li C, Zhong H, Cao Z, Wang F, Sun Y, Wang L, Hou J, Zhang C, Yang M, Ding Y, Yao Y, Li P, Zhu YG (2025) *Aspergillus* mycotoxins: The major food contaminants. Advanced Science (Weinheim, Baden-Württemberg, Germany) 12: e2412757. https://doi.org/10.1002/advs.202412757

Zhang ZF, Zhou SY, Eurwilaichitr L, Ingsriswang S, Raza M, Chen Q, Zhao P, Liu F, Cai L (2021) Culturable mycobiota from Karst caves in China II, with descriptions of 33 new species. Fungal Diversity 106: 29–136. https://doi.org/10.1007/s13225-020-00453-7

Supplementary material 1

Maximum likelihood phylogenies

Authors: Lu-Yao Peng, Wen-Ying Zhuang, Xin-Cun Wang

Data type: zip

Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/mycokeys.124.172775.suppl1